WHWTs with CAD and 26 control WHWTs were genotyped. Microsatellite alleles, their sizes and distribution in affected and control Boxer and WHWT animals are shown in Table 1. The microsatellites were informative, and the alleles were equally distributed among cases and controls in both breeds. No statistically significant difference in allele distribution was observed (data not shown). Plausible haplotypes were computed for the genotypes using PHASE version 2.1.[6] Based on the observed microsatellite alleles, 19 and 14 possible haplotypes were generated in the PHASE analysis yet five and eight haplotypes were observed in Boxers and WHWTs, respectively. Statistical evaluations were performed by chi-square analyses and showed alleles in significant LD. The distribution of these marker alleles defined haplotype blocks at the *KLK7* locus (Table 2). None of the observed haplotypes showed genetic association in either CAD-affected dogs or unaffected dogs.

Gene annotation: The nucleotide sequence of human *KLK7* (AF166330) was used for comparative analyses against the whole-genome Boxer sequence.[5] The dog ortholog was identified using the ENSEMBL v37– Feb 2006[7] release and aligned to the human sequence with a modified version of the NCBI bl2seq programme (E. Bongcam-Rudloff and A. Nistér, unpublished data; Fig. S1). Canine *KLK7* is 3.1 kb, contains five exons (with an estimated transcript size of 774 bp) and is located on CFA1 at position 108 156 349–108 159 472.

Conclusions: We demonstrate here through association analysis that it is highly unlikely that *KLK7* is a disease gene for CAD development in Boxer and WHWT.

Acknowledgements: We thank Leif Andersson and Örjan Carlborg for advice and suggestions and Ulla Gustafson for technical support. The study was supported by strategic funding from the Swedish University of Agricultural Sciences and by the Agria Insurance Company Research Foundation.

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Supplementary Material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2006.01537.x

Table S1 PCR primers and positions of *KLK7* microsatellites.

Figure S1 Human *KLK7* aligned to dog orthologs using the modified bl2seq program.

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doi:10.1111/j.1365-2052.2006.01536.x

Linkage and RH mapping of 10 genes to a QTL region for fatness and muscling traits on pig chromosome X

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Accepted for publication 1 October 2006

Source/description: In this study, 10 genes located on human chromosome region Xq13.1–Xq24 and homologous to a QTL region for fatness and body conformation traits in pigs (http://www.animalgenome.org/QTLdb/pig.html) were mapped using at least one of the following resources: the U.S. Meat Animal Research Center (USMARC) backcross family,[1] the University of Hohenheim Wild Boar × Meishan (W×M) and Meishan × Piétrain (M×P) F_2 families[2] and the INRA-University of Minnesota porcine radiation hybrid $(IMpRH)_{7000}$ panel.[3]

Primer design and PCR conditions: PCR primers for amplification of porcine genomic DNA were designed from the porcine sequence (in the case of *HTR2C*) or the orthologous human sequences. The PCR products were synthesized in a PTC-200 DNA thermal cycler (MJ Research Inc. Watertown, MA, USA) using 100 ng genomic DNA from each of six founder animals (2 Meishans, 2 Piétrains and 2 European Wild Boars) under conditions given in Table S1. The fragments were sequenced, analysed for polymorphisms, and deposited into the EMBL database (accession numbers are given in Table 1). Primers for RH mapping (Table S1) were designed from the porcine sequences generated in this study, and the PCR products were checked for specificity with porcine and hamster genomic DNA.

Radiation hybrid mapping: Radiation hybrid mapping of nine genes was performed by amplifying markers across the IMpRH panel using the primers and PCR conditions given in Table S1. Results including retention frequencies, nearest linked markers, distances on the existing porcine RH map[5] and LOD scores from two-point analyses (http://imprh.toulouse.inra.fr)[4] are provided in Table 1.

Linkage mapping: PCR-RFLP assays were prepared for SNPs of seven genes using the restriction enzymes listed in Table S1. These SNPs were genotyped in 69 unrelated pigs of eight breeds for estimation of allele frequencies (Table S2) and in all animals of the reference families. Genotyping of *SERPINA7* in the Uni-

Table 1 Results of RH and linkage mapping of 10 genes to the porcine chromosome X region harbouring QTL for fatness and muscling traits.

				RH mapping				Position on linkage maps (cM)		
Gene/ marker	Location on HSA X (Mbp)	Porcine sequence	SNP	Retention frequency (%)	Closest marker	Distance (cR)	LOD score	USDA-USMARC	W×M 18 markers	M×P 17 markers
SW259	–	–	–	–	–	–	–	74.4	85.7	65.9
RPS4X	71.40	AJ429141	g.138T>C	35	SW1835	0.73	5.07	74.4	86.3	66.8
XIST	72.95	AJ429140	g.769T>G	41	*SW1835*	0.41	10.54	74.4	86.3	–
POU3F4	82.64	AJ429271	g.248G>A	26	*SW1994*	0.70	5.35	74.4	86.3	66.8
NOX1	99.98	AJ429272	–	37	*SW154*	0.14	20.52	–	–	–
CENPI	100.24	AJ784838	–	30	*SW154*	0.24	15.45	–	–	–
SERPINA7	105.16	AJ293944	–	35	*SW1426*	0.55	7.62	75.5	86.6	68.1
ACSL4	106.69	AJ785784	g.388G>C	39	*SW1426*	0.70	5.39	80.0	90.0	72.7
CAPN6	**110.37**	AJ429142	g.267A>C	47	*SW1426*	0.52	7.85	81.0	95.6	79.3
PAK3	**110.22**	AJ429269	g.440T>C	37	*SW1943*	0.70	5.31	82.5	96.5	80.0
HTR2C	113.72	AF188614	g.142G>C	–	–	–	–	87.4	–	–
SW1943	–	–	–	–	–	–	–	87.4	106.1	95.6

–, no data obtained. Locations of genes on human chromosome X with inverted order in pigs are given in bold.

versity of Hohenheim W×M and M×P families was described elsewere[6]. Multipoint linkage analysis of the families was performed using CRI-MAP version 2.4[7]. Seven genes (*RPS4X*, *XIST*, *POU3F4*, *ACSL4*, *CAPN6*, *PAK3* and *HTR2C*) were linkage-mapped to the porcine X chromosome between *SW259* and *SW1943* on the current USMARC linkage map; seven (*RPS4X*, *XIST*, *POU3F4*, *SERPINA7*, *ACSL4*, *CAPN6* and *PAK3*) were placed on the University Hohenheim W×M linkage map; and six (*XIST*, *POU3F4*, *SERPINA7*, *ACSL4*, *CAPN6* and *PAK3*) genes were located on the University of Hohenheim M×P linkage map (Table 1). The positions of *SW259*, *SW1943* and *SERPINA7* were previously published.[6,8]

Comments: Seven genes were newly assigned on the chromosome X linkage map between *SW259* and *SW1943*, which also contains a QTL for fatness and muscling traits in the W×M and M×P families respectively.[9,10] Four genes (*SW259*, *RPS4X*, *XIST* and *POU3F4*) located in a region with low recombination[11] in the USMARC pedigree (86 progeny) were mapped to position 74.4 cM. Using the larger University of Hohenheim W×M (335 F_2 animals) and M×P (316 F_2 animals) pedigrees, only *SW259* could be separated from this block. Nine genes (the seven genes on the linkage map, *NOX1* and *CENPI*) were also mapped using the $IMpRH_{7000}$ panel. Five of them (*RPS4X*, *XIST*, *POU3F4*, *NOX1* and *PAK3*) were new assignments on the RH map. The order of the closest markers to which the genes were linked on the $IMpRH_{7000}$ panel is based on data published elsewhere.[12] Comparison of porcine order with gene order on the human X chromosome (NCBI Build 6.1, last visit 25th June 2006) showed an inversion of the porcine genes *CAPN6* and *PAK3* (Table 1). This inversion will be confirmed on the next-generation, high-density porcine RH map.

Acknowledgements: Technical assistance of Ms Markéta Hančová is appreciated. This work was supported by the Institutional Research Plan of the IAPG AS CR (AV0Z50450515) and the Czech Science Foundation (grant 523/04/0106).

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Supplementary Material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2006.01536.x

Table S1 Source sequences, location of genes on human cytogenetic map, primers, annealing temperatures (T_a) and amplicon sizes of PCR products used for RH and linkage mapping and restriction enzymes used for SNP typing.

Table S2 SNP allele frequencies for *RPS4X*, *XIST*, *POU3F4*, *ACSL4*, *CAPN6*, *PAK3* and *HTR2C* in eight pig breeds.

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